Flora W. Patterson

BULLETIN No. 251. OCTOBER, 1904.

New York Agricultural Experiment Station.

GENEVA, N. Y.

IMPERIAL BUREAU 13 JUL 1921 OF MYCOLOGY

VITALITY OF THE CABBAGE BLACK ROT GERM ON CABBAGE SEED.

H. A. HARDING, F. C. STEWART AND M. J. PRUCHA.

PUBLISHED BY THE STATION.

BOARD OF CONTROL.

GOVERNOR BENJAMIN B. ODELL, Jr., Albany.
STEPHEN H. HAMMOND, Geneva.
FREDERICK C. SCHRAUB, Lowville.
LYMAN P. HAVILAND, Camden.
EDGAR G. DUSENBURY, Portville.
JENS JENSEN, Binghamton.
THOMAS B. WILSON, Halls Corners.
MILO H. OLIN, Perry.
IRVING ROUSE, Rochester.
CHARLES W. WARD, Queens.

OFFICERS OF THE BOARD.

STEPHEN H. HAMMOND, *President.*
WILLIAM O'HANLON, *Secretary and Treasurer.*

EXECUTIVE COMMITTEE.

STEPHEN H. HAMMOND,
FREDERICK C. SCHRAUB,
LYMAN P. HAVILAND,
——— ———,
THOMAS B. WILSON.

STATION STAFF.

WHITMAN H. JORDAN, Sc. D., *Director.*

GEORGE W. CHURCHILL, *Agriculturist and Superintendent of Labor.*
WILLIAM P. WHEELER, *First Assistant (Animal Industry).*
FRED C. STEWART, M.S., *Botanist.*
HARRY J. EUSTACE, B.S., *Assistant Botanist.*
LUCIUS L. VAN SLYKE, PH.D., *Chemist.*
EDWIN B. HART, B.S., *Associate Chemist.*
*WILLIAM H. ANDREWS, B.S.,
FREDERICK D. FULLER, B.S.,
*CHARLES W. MUDGE, B.S.,
ANDREW J. PATTEN, B.S.,
*FRANK A. URNER, A.B., *Assistant Chemists.*
HARRY A. HARDING, M.S., *Dairy Bacteriologist.*
MARTIN J. PRUCHA, PH. B., *Assistant Bacteriologist.*
GEORGE A. SMITH, *Dairy Expert.*
FRANK H. HALL, B.S., *Editor and Librarian.*
PERCIVAL J. PARROTT, M.A., *Entomologist.*
HAROLD E. HODGKISS, B.S., *Assistant Entomologist.*
SPENCER A. BEACH, M.S., *Horticulturist.*
NATHANIEL O. BOOTH, B. AGR., *Assistant Horticulturist.*
ORRIN M. TAYLOR, *Foreman in Horticulture.*
† F. ATWOOD SIRRINE, M.S., *Special Agent.*
FRANK E. NEWTON,
JENNIE TERWILLIGER, *Clerks and Stenographers.*

ADIN H. HORTON, *Computer.*

Address all correspondence, not to individual members of the staff, but to the NEW YORK AGRICULTURAL EXPERIMENT STATION, GENEVA, N. Y.

The Bulletins published by the Station will be sent free to any farmer applying for them.

---

* Connected with Fertilizer Control.
† In Second Judicial Department.

BULLETIN No. 251.

VITALITY OF THE CABBAGE BLACK ROT GERM ON CABBAGE SEED.[1]

H. A. HARDING, F. C. STEWART AND M. J. PRUCHA.

SUMMARY.

Black rot of cabbage is a destructive bacterial disease caused by *Pseudomonas campestris* (Pam.) Smith. No satisfactory method of controlling the disease in the field has yet been found. Concerning the ability of the disease germs to survive the winter on the seed, there has been a difference of opinion. The present bulletin gives an account of some investigations bearing on this point.

The conclusion is that much of the cabbage seed on the market is contaminated with germs of the black rot disease and that some of these germs may survive the winter and become a source of infection to the young cabbage plants.

As a precautionary measure, it is advised that all cabbage seed be disinfected before sowing, by soaking for fifteen minutes in a 1–1000 corrosive sublimate solution or in formalin, one pound to thirty gallons. It is not expected that this treatment will prevent either leaf or root infection in infected soils; but it may be safely relied upon to prevent all danger from infected seed. It will not injure the germination.

[1] A preliminary note on this investigation was published in *Science*, N. S. **20**: 55. July 8, 1904.

INTRODUCTION.

Black rot in cabbage is primarily a trouble of the fibro-vascular bundles. During the progress of the disease these bundles become dark brown or black. On cutting across the petiole of a diseased leaf the affected bundles are seen as dark points. When so many of the bundles are affected as to cut off the supply of water to a leaf the blade dries up and resembles a piece of brown parchment, the blackened veinlets standing out sharply against the brown background. As the leaves in the head become affected they decay, producing a dark, vile-smelling mass.

This trouble was brought to the attention of pathologists at the time of an epidemic when the loss in infected fields was often total and consequently an exaggerated idea of the average fatality of the disease arose. Study extended over a number of seasons has shown that the true epidemic aspect appears only under unusual combinations of several factors, prominent among which are high temperature and abundant moisture. Under usual conditions a majority of the plants in a field may be infected and yet mature a considerable crop although a smaller one than would have been otherwise produced. In the latter case the disease may pass unnoticed except by those familiar with its appearance.

The black rot of cabbage is caused by *Pseudomonas campestris* (Pam.) Smith, a species of bacterium which attacks several species of the Cruciferæ. This germ is widespread in the United States east of the Mississippi River; and in September, 1902, Prof. W. Paddock sent us a diseased cabbage from Colorado affected with what he believed to be black rot. In a letter accompaning the specimens he stated that this disease had been destructive in Colorado in 1901 and 1902. We established the correctness of his diagnosis by isolating *P. campestris* and determining its pathogenicity upon young cabbages in the greenhouse. While the presence of *P. campestris* in Europe[2] was not reported until 1899, the prompt-

[2] Harding, H. A. Die schwarze Fäulnis des Kohls und verwandter Pflanzen, eine in Europa weit verbreitete bakterielle Pflanzenkrankheit. *Cent. f. Bakt.*, etc., II. Abt., **6**: 305–313. 1900.

ness with which this report has been confirmed by observers in England [3], Holland [4], Denmark [5], Austria [6] and Switzerland [7] suggests that it is there widespread and of long standing.

As we have noted in an earlier bulletin [8] the germs causing the disease sometimes gain an entrance through the broken roots, at the time of transplanting. This avenue of infection is most used during the early life of the plants. Later the germs commonly enter through the water pores at the margins of the leaves. Accordingly, the regular removal of the diseased leaves has been recommended by different writers as a method of prevention but we have carefully tested this method and found it to be a complete failure. [9]

Since the disease is annually the cause of considerable loss to the cabbage growers of the State it has been thought best to continue our study of the disease in the hopes of finding successful means of controlling it. This publication deals with the vitality of the disease germs in a dry condition, as well as their ability to survive upon cabbage seed, together with some suggestions as to protection against this danger.

A belief in the transmission of the disease by means of the seed has been held by many large growers and the subject has been mentioned by other writers, but so far as we can learn this is the first attempt to determine experimentally the correctness of this view. While we believe that the facts to be presented have been established in a reliable manner there are so many more related points of interest and importance which

---

[3] Potter, M. C. On the Brown Rot'of the Swedish Turnip. *Jour. Boarp of Agr.*, **10**: 314. 1903.

[4] van Hall, C. J. J. Twee Bacteriënziekten. *Tijdsch. over Plantenziekten*, **6**: 169–177. 1900.

[5] Rostrup, E. 17. Oversigt over Landbrugsplanternes Sygdomme i 1900. Sep.-Abr aus *Tidsskrift for Landbrugets Planteavl*, **8**: 109–128. Kjöbenhavn, 1901. [Ref. *Zeit. f. Pflanzenkr.*, **12**: 293. 1902.]

[6] Hecke, Ludwig. Eine Bakteriose des Kohlrabi. *Zeitschrift für das landwirthschaftliche Versuchswesen in Oesterreich*, 1901. S. 469. [Ref. *Zeit. f. Pflanzenkr.*, **11**: 273. 1901.]

[7] Brenner, W. Die Schwarzfäule des Kohls. *Cent. f. Bakt.*, etc., II. Abt., **12**: 725–735. 1904.

[8] New York Agr. Exp. Sta. Bul. 232: 60–61. 1903.

[9] Loc. cit., pp. 49–62.

have not yet been solved that we had not intended to present the subject until the latter had been studied. However, the facts which we have determined seem to have such a practical bearing that we have presented them in this preliminary bulletin trusting to enlarge upon their relation to the whole problem in a later publication.

BLACK ROT IN FIELDS OF SEED CABBAGE.

SOURCE OF CABBAGE SEED.

At the beginning of the last century, Holland and Denmark furnished the bulk of our cabbage seed. The production at home has increased steadily and now the seed imported represents a small fraction of the total supply and that largely of the cheaper grade.

Considerable cabbage seed is now produced around Puget Sound, but for more than a quarter of a century Long Island has been an important source of supply. The soil and climate of portions of the island seem admirably adapted to this crop. While many market gardeners at the western end of the island produce seed in limited quantities, the commercial phase of the industry is most developed at the eastern end in the region about Cutchogue. Here, cabbage seed fields of several acres are common and cabbage seed is one of the staple crops.

METHOD OF SEED GROWING.

On Long Island, stock seed of the desired variety is grown under the immediate supervision of the large seedsmen. This is furnished to the farmers who produce the commercial seed under contract. The seed is sown so that the plants are ready to transplant early in August and do not reach maturity until cold weather. Early in November they are buried in shallow trenches to be set out again the following April. The flowers appear in May and the seed is ripe in July. It was formerly threshed by hand but a small threshing machine is now used by the larger growers.

DISEASES OF THE PLANTS.

While growing in the field the first season, the plants are subject to all of the ordinary cabbage diseases and in some seasons

black rot has been so destructive as to make the crop practically a failure. While in the trenches in the winter a variable number of the plants rot. This is in small part the result of the black rot but is more largely due to soft rot caused by an entirely different organism identical with or closely related to *B. carotovorous* Jones.[10] A prominent seedsman who has been in the business extensively on the Island for over a quarter of a century estimates the annual loss, from rot, at twenty-five per ct. of the crop. In some cases the crop is a total loss.

This soft rot occurring in the trenches or appearing after the plants have been reset has been under observation for a number of years and we hope to present some of the results of our observation in a later bulletin.

BLACK ROT GERMS PASS THE WINTER IN CABBAGE PLANTS.

As has been stated, cabbages intended for raising seed the following season are placed in shallow trenches at the approach of freezing weather and covered with six to twelve inches of soil. Here they are frozen in and remain until spring.

At the time of opening the trenches in the spring we have on a number of occasions collected plants showing black bundles and taken them to the laboratory for study. From these plants by culture methods we have obtained *P. campestris*, it often being the only organism present in the darkened bundles. In order to avoid possible errors in recognizing the germ, cultures have been inoculated into healthy young cabbage and cauliflower plants with all precautions necessary to prevent contamination. After such inoculation black rot has appeared in two to three weeks. Check plants treated in the same manner except that they were punctured with a sterile needle have always remained healthy.

From this it is evident that the black rot germs are present in some of the plants at the time they are set out in the spring and that they are in condition to continue their attack upon the plants.

---

[10] Harding, H. A. & Stewart, F. C. A Bacterial Soft Rot of Certain Cruciferous Plants and *Amorphophallus simlense*. *Science*, N. S., **16** : 314–315. Aug. 22, 1902.

OBSERVATIONS IN THE FIELD.

May 26, 1899, six fields of seed cabbage just in bloom near Cutchogue were examined. In all the fields many plants showed the characteristic blackening of the fine veins of the leaves and in some cases so many of the bundles in the stem were affected as to cause the plant to wilt. Soft rot was sometimes present in the plants suffering from black rot.

Although it is possible to recognize the disease in the field with great certainty, in order to settle the matter beyond question plants were taken to the laboratory and cultures made from the blackened bundles. *P. campestris* was isolated from four such plants and the identity of the cultures was further strengthened by inoculating in each case into healthy cabbage plants. Each culture produced the characteristic black rot when thus inoculated into healthy cabbage plants while other healthy plants grown along side and treated as controls did not take the disease.

Accordingly the fact that *P. campestris*, the cause of black rot, was present in these seed cabbage plants at blooming time is settled beyond question. The observations of each succeeding season show that while the amount of black rot present varies considerably with different seasons the disease is never absent from any considerable area.

DISEASE GERMS ON THE SEED.

ISOLATION OF GERM FROM SEED PLANTS.

In June, 1900, we selected and marked four seed-bearing plants in the fields near Cutchogue, which were affected with black rot. When the seed was ripe the plants were collected by W. A. Fleet, inclosed separately in large paper bags and expressed to us. Each plant was threshed separately and a small portion of the seed soaked in sterile water. Cultures were made from this water and small portions of it were also injected into young healthy cabbage plants in the greenhouse with the usual precautions to prevent the entrance of germs from other sources. The amount of water which it is possible to inject into growing tissue is small and the production of disease in six out of twelve

experimental plants is surprising. These results demonstrated the presence of *P. campestris* on the seed of three of the four seed plants examined.

The cultures made from the same water which was used in the direct inoculation of the young plants showed the presence of *P. campestris* in the case of three of the seed plants. That the colonies were really *P. campestris* was settled by inoculation with all necessary precaution into healthy young plants in the greenhouse. Black rot was produced by the use of pure cultures derived from the seed of these three different seed plants.

On comparing the results from these two methods of testing the infectiousness of the seed it was seen that the germs had been isolated by cultures from the seed of the plant which had failed to show results from the direct inoculation. Accordingly, we have a complete demonstration that the germs of black rot were present on the seed of all four of these diseased cabbage seed plants at the time of harvesting.

INTERPRETATION OF THESE RESULTS.

Concerning any experimental work it is always proper to consider in how far the conditions of the experiment have deviated from the normal and in how far the results represent actual conditions.

In 1900, up to the time of our visit in June, the conditions had not been favorable for an epidemic of black rot and the fields around Cutchogue were unusually free from evidence of this disease. All of the plants marked were later shipped us, indicating that there was little destruction after that date. The plants were fairly well developed in spite of the disease and undoubtedly would have been harvested under ordinary conditions. The plants themselves were, then, a normal part of the harvest except that their chance of carrying the disease was above the average of all the plants in the field.

The threshing operations differed somewhat from the normal. In the ordinary method the seed from a large number of plants is pounded out by hand until a heap of seed, pods and fine branches is accumulated, or the whole plant is run through a threshing machine which reduces the dry branches almost to chaff. In either case the slightly oily seeds are exposed to the dust from

the crushed diseased bundles which are fairly sure to be present in considerable numbers.

The seed plants we examined were held until they were thoroughly dry, when they were threshed and the seed from each plant cleaned separately with the least possible breaking of branches or exposure to dust. In this particular they were exposed to less chance of contamination than is the seed from healthy plants under ordinary conditions.

After a careful study of the conditions attending the growth and harvesting of seed cabbage there can be no doubt that there is at least a small number of *P. campestris* on practically all the seed produced on Long Island.

While this is in some respects an undesirable state of affairs it should not be forgotten that it is one for which neither the grower nor the seedsman can be justly held responsible. For financial reasons most growers do reject, at the time of resetting, all plants evidently affected with black rot since they have found that such plants often fail to produce seed. However, from the nature of the disease they are not able to detect the less advanced cases nor can they prevent infection occurring during the second season.

The remedy does not lie in obtaining seed from other sources since there is every reason to believe that the other sources of seed are equally affected. It lies rather, as will be later shown (page 192), within the control of the individual purchaser.

VITALITY OF GERMS ON SEED.

Even though large numbers of *P. campestris* are attached to the seed at the time of harvest it does not necessarily follow that they will survive long enough to affect the succeeding crop.

P. campestris does not form spores and consequently is not well fitted to withstand adverse conditions for a long period. Upon the surface of the smooth, hard, cabbage seed, food is scarce; and moisture, which is so necessary to the life of the germs, is reduced to a minimum. The close dependence of *P. campestris* upon moisture is shown by its rapid destruction when exposed to dessication.

EFFECT OF DESSICATION ON *P. campestris*.

This point was previously tested by one of us (H) at the Wisconsin Station[11] by allowing a small drop of fresh bouillon culture of the organism to dry upon sterile cover slips, which were kept in darkness at room temperature.

In the limited number of tests which were made at that time the germs all died within 45 hours.

After the somewhat surprising results from our tests of the vitality of *P. campestris* upon cabbage seed, the tests upon cover glasses were repeated using the same organism which had been used to infect the seeds and carrying on the test under conditions which would be closely comparable to those to which the seeds were exposed.

The cultures used at Wisconsin had been grown in the laboratory about a year at the time of the test and the results may be open to some objections on that score. In order to be sure in the present instance that the culture was as nearly as possible in its natural condition it was first inoculated into a cabbage plant. There it produced the characteristic lesions of black rot. From these plants the culture was isolated and its purity determined.

The growth upon agar slope, two to three days old, was then rubbed up in sterile water as thoroughly as possible and the emulsion filtered through paper. This gave a faintly milky solution containing a very great number of individuals but free from lumps and large masses of germs.

Small drops of this liquid were transferred to sterile cover slips placed in sterile Petri dishes. These drops dried up within a few minutes leaving a film of germs upon each cover slip. The Petri dishes each containing five cover slips, were then wrapped in paper and placed in a closed drawer at a temperature of about 70° F. (21° C.).

Each day five coverslips were transferred each to a sterile Petri dish and melted agar added. At the end of several days transfers were made from the resulting colonies to sterile potato upon which *P. campestris* produces most characteristic growth. Pure cultures were also preserved for further identification.

The results of three such tests may be summarized as follows :

[11] Wis. Agr. Exp. Sta. Bul. 65 : 19. 1898.

During the first two days a large part of the germs are destroyed and by the end of the third day few survive. From the third to the tenth day an occasional germ is found alive but we have not found one alive after ten days. In many cases they were all dead on a coverslip before the end of the third day.

PLAN OF TEST ON CABBAGE SEED.

In view of the above results it seemed hardly probable that the germs on the seed would survive long enough to be a source of danger. To settle this point it was decided to infect some seed heavily and observe the length of time that the germs would survive.

In the test of April 29, 1903, a vigorous growth of *P. campestris* two days old on agar slope was covered with ten cubic centimeters of water and thoroughly stirred into the water. This emulsion was filtered through paper to remove the larger solid particles and 5 cc. of the filtrate poured over 260 cabbage seeds (var. Early Jersey Wakefield). After the seeds had soaked in the germ-impregnated water for a few minutes they were spread out to dry for about four hours. They were then thoroughly dry. Five seeds were placed in each of 52 sterile test tubes plugged with cotton. Twenty-six tubes were left in this condition while the other twenty-six were sealed by paraffining the cotton plugs. The whole 52 tubes were then put into covered pasteboard boxes and stored in a room which was kept at a temperature ranging from 60°–70° F. (16–21° C.). The object of paraffining a portion of the tubes was to prevent excessive drying. By treating the seeds in these different ways it was hoped to approximate the conditions at the outside and at the interior of commercial packages of seed held in storage.

SOURCE AND PATHOGENICITY OF CULTURE.

The culture of *P. campestris* which was used in this test was isolated in March, 1902, from a Long Island seed cabbage which had already passed the winter in the trenches.

To be certain that neither its exposure to low temperatures during the winter nor its subsequent long cultivation in the laboratory had destroyed its pathogenicity it was inoculated with proper precautions into two young healthy cabbage plants in the greenhouse, April 6, 1903. Ten days later the first signs of

PLATE I.—INOCULATED AND UNINOCULATED CABBAGE PLANTS.

AT THE LEFT, A PLANT SUFFERING FROM A SEVERE ATTACK OF BLACK ROT ONE MONTH AFTER INOCULATION WITH *P. campestris* WHICH WAS ISOLATED FROM CABBAGE SEED HELD IN DRY CONDITION FOR ELEVEN MONTHS. AT THE RIGHT, A HEALTHY CONTROL PLANT

black rot were showing and within a few days both plants developed unmistakable evidence of this disease. The check plants remained healthy.

EXAMINATION OF THE SEED.

Tubes containing 5 seeds each were examined at intervals of about a month. One to two cc. of sterile water was added to each tube and allowed to stand over night. On the following morning the water and seeds were distributed among three lactose agar plates. From these plates the resulting colonies were removed and studied.

A detailed statement of the dates and results of the various examinations is given in the accompanying table.

TABLE I.—EXAMINATION OF TUBES CONTAINING ARTIFICIALLY INFECTED CABBAGE SEED.

Date.	Interval.	Tubes examined.		Tubes containing *P. campestris*.	
		Paraffined.	Not paraffined.	Paraffined.	Not paraffined.
1903	Months.	*No.*	*No.*	*No.*	*No.*
April 29	Experiment started.				
June 2	1	3	3	2	2
July 1	2	3	3	1	2
Aug. 1	3	1	1	0	0
Sept. 4	4	3	3	1	1
Oct. 2	5	3	3	2	3
Nov. 7	6	3	3	3	3
Dec. 9	7	3	3	2	2
1904.					
Jan. 14	8½	1	1	0	0
Jan. 28	9	1	1	0	0
Feb. 25	10	3	3	2	3
April 4	11	2	2	1	1
Total		26	26	14	17

Attention should be called to the fact that in these tests we did not determine the number of germs which survived in any case but merely the fact that *P. campestris* was alive in certain tubes. A quantitative determination would have been desirable but in the nature of the case was difficult. The germs became so closely

attached to the seeds in the long drying that when the seeds were placed directly into the agar the germs could not be dislodged by shaking and later produced a confused growth around the imbedded seeds. Grinding of the seeds was attempted but their texture was such that they were not reduced to sufficiently fine particles and the resulting growth was largely in clumps surrounding the fragments of seeds.

We did not think it desirable to sterilize the seeds before applying the cultures of *P. campestris* as such treatment would probably leave the surface of the seed in an abnormal condition and vitiate the experiment. Accordingly, the growths around the seeds were often a mixture in which *P. campestris* was compelled to struggle for existence and from which it must be isolated by sub-cultures. An earlier attempt to determine the vitality of *P. campestris* on cabbage seed failed because we added the seed to be tested to the agar without previous soaking.

A positive result in this experiment indicates with certainty that *P. campestris* was alive upon the seed but a negative result does not make it certain that they were all dead. On practically all of the seeds there were germs present other than *P. campestris*. In some cases these germs found the watery solution to which they were exposed some hours so satisfactory that they developed rapidly and overran the resulting cultures so completely that a recognition of *P. campestris* would have been impossible even though it had been present in moderate numbers.

PATHOGENICITY OF GERMS ISOLATED FROM CABBAGE SEED.

The ability to produce disease is a function of germs which is most readily affected by unfavorable conditions. It would not have been surprising if the *P. campestris* which had remained over winter on the dry cabbage seed were so weakened by this exposure as to be incapable of attacking the tissue of healthy cabbage plants.

Cultures of the germs which had been found on the seed at the various examinations were preserved and on March 25, 1904, the pathogenicity of a number of these cultures was tested by inoculating healthy cabbage plants. These plants were grown in the greenhouse and were inoculated with all precautions necessary to prevent the entrance of other germs. Several check

plants were treated in the same way in all respects except that they were pierced with a sterile needle instead of one dipped into a bacterial culture. Two plants were inoculated with each culture and eight cultures were tested representing two tubes from the November, three tubes from the December, and three tubes from the February examination. Two cultures were from tubes which had been paraffined and six from unparaffined tubes.

April 15, the characteristic symptoms of black rot were showing in fourteen of the sixteen inoculated plants. May 7, the disease was very evident in all of the inoculated plants. In order to complete the proof that the diseased condition of the plants was really due to the germ inoculated, cultures were made from the diseased tissue in three plants representing a like number of the original eight cultures and *P. campestris* found in pure culture. The check plants which had grown beside the inoculated ones all remained healthy.

April 26, each of the two cultures obtained from the examinations of April 4, were inoculated into two young cabbage plants in the greenhouse with all necessary precautions. The plants were growing rapidly and the temperature in the greenhouse was high. May 3, the disease was showing in two of the inoculated plants and May 7, it was evident in all four of them. Cultures were made from the tissue of these plants and *P. campestris* obtained. The two control plants remained healthy. The accompanying plate (Plate I) shows one of these control plants as well as one inoculated with the culture of *P. campestris*. A number of leaves have already been destroyed with the characteristic lesions of black rot. The photograph was taken one month after the inoculation.

REPETITION OF THE TEST ON CABBAGE SEED.

Oct. 17, 1903, a second lot of cabbage seed was infected as nearly as possible in the same manner as in the first test using the same strain of *P. campestris*. In this test ten seeds were placed in each test tube.

We were unfortunate in this case in selecting cabbage seed which was infected with a resistant and rapid growing bacillus. When the examination of this seed was begun at the eighth month the abundant growth of this form covered the plates so

completely that no *P. campestris* could be found. The method of examination was later changed so as to leave the seeds in the water only a few hours. With this modification we have obtained *P. campestris* at the end of 8½ and 9½ months respectively and the examination will be continued at later intervals.

SEED DISINFECTION.

From what has already been shown concerning the prevalence of black rot in the seed cabbage fields, the presence of the disease germs on the seed and their ability to remain alive there, it is seen that the commercial seed is a factor in the spread of the disease germs. A simple and effective method of destroying these germs is much to be desired.

SOAKING A SATISFACTORY METHOD.

Soaking seed potatoes, oats, wheat and barley in disinfecting solutions to free them from disease spores is common agricultural practice. When small quantities of seed are to be treated, as is the case with cabbage, soaking for a short time in a solution combines simplicity with thoroughness in a satisfactory manner.

In this work, the destruction of the disease germs without injury to the germination of the seed is the point to be attained. Fortunately, *P. campestris* does not form spores and is quickly killed by weak solutions of common disinfectants, a 0.5 per ct. solution of lysol[12] destroying it in one minute. Since the time for sowing cabbage seed is about the same as that for planting potatoes we have tested the solutions of corrosive sublimate and formalin commonly used upon potatoes with regard to their effects upon the germination of cabbage seed.

EFFECT OF CORROSIVE SUBLIMATE ON GERMINATION.

It was first attempted to determine what strength of corrosive sublimate solution can be borne by cabbage seeds without injury to their germination. Three lots of seed, one hundred seeds each, were placed for five minutes in corrosive sublimate solutions having a strength of 1–500, 1–1000, and 1–2000. A fourth lot of one hundred seeds was soaked in distilled water for five min-

[12] Wis. Agr. Exp. Sta. Bul. 65 : 19. 1898.

utes as a control. The seeds were sown in pots in the greenhouse on April 7 and covered with soil to a depth of three-eighths of an inch. On April 17 the number of plants showing above ground was as follows :—Control, 75 ; corrosive sublimate 1–500, 74 ; corrosive sublimate 1–1000, 80 ; corrosive sublimate 1–2000, 70. The treated seed gave seedlings as vigorous as those from the control. Apparently, there was no injury in any case.

As 1–1000 is the strength of corrosive sublimate used on seed potatoes it was next undertaken to determine the length of time that cabbage seed may be exposed to it without injury. April 22 one hundred cabbage seeds were placed in 1–1000 solutions of corrosive sublimate for periods of 15, 30 and 60 minutes. The same number of seeds were soaked one hour in distilled water. After treatment, the seeds were planted as before.

May 3 the seedlings were counted with the following result :— Control, 69 ; corrosive sublimate 15 minutes, 73 ; corrosive sublimate 30 minutes, 82 ; corrosive sublimate one hour, 71. In this experiment the plants from untreated seed seemed a trifle more vigorous than those from treated seed. However, in view of the fact that the percentage of germination was not lowered in any case it seems likely that the greater vigor of the control plants was due to some other cause than the treatment.

Later, a pound of cabbage seed was soaked for fifteen minutes in 1–1000 corrosive sublimate solution apparently without injury. The seed germinated satisfactorily and the plants grew thriftily.

EFFECT OF FORMALIN ON GERMINATION.

In order to determine whether cabbage seed may be disinfected with formalin without injury to the germination the following experiment was made : Ten cubic centimeters of formalin (40 per ct. formaldehyde) was mixed with 2400 cubic centimeters of water. In this solution eight lots of cabbage seed, 100 seeds in each lot, were soaked for different lengths of time ; two lots, 15 minutes ; two lots, 30 minutes ; two lots, one hour ; two lots, two hours. Two other lots were left untreated for a check. As soon as the treated seeds were dried they were sown in boxes in the greenhouse. This was done September 23. On October 10 the plants from each lot of seed were counted. The seed treated fifteen minutes gave an average germination of 38 per

ct. ; 30 minutes, 32.5 per ct. ; one hour, 24.5 per ct.; two hours, 14 per ct.; untreated, 34 per ct. The seedlings appeared equally vigorous in all lots except those treated for two hours. It seems likely that the two-hour treatment was somewhat injurious, but the experiment must be repeated before a positive statement to that effect can be made. The 15-minute treatment appeared to do no harm.

The strength of the formalin solution used in this experiment was the same as that generally recommended for use in treating potatoes to prevent scab, namely, one pint or pound to thirty gallons of water.

DIRECTIONS FOR SEED DISINFECTION.

Cabbage and cauliflower seed may be disinfected either with corrosive sublimate or with formalin.

If corrosive sublimate is used, the strength of the solution should be one part of corrosive sublimate to one thousand parts of water. The most convenient method of preparing this solution is to use the corrosive sublimate tablets sold by druggists for making disinfecting solutions. One tablet, costing one cent, is sufficient to make a pint of solution which is about the quantity required to treat one pound of cabbage seed. The seed should be soaked in this solution for fifteen minutes and then spread out to dry.

If formalin is used the strength of the solution should be one part of formalin (40 per ct. formaldehyde) to 240 parts of water and the seed soaked fifteen minutes.

Both with corrosive sublimate and formalin the strength of solution recommended is approximately the same as is generally used in treating seed potatoes to prevent scab ; namely, two and one-fourth ounces of corrosive sublimate to fifteen gallons of water or one pint of formalin in thirty gallons of water. Since the usual time of sowing cabbage seed is about the same as that for planting potatoes it may often happen that the solution required for disinfecting the cabbage seed may be taken from that prepared for treating potatoes, thus avoiding the bother of preparing a small quantity especially for the cabbage seed.

While it appears that cabbage seed is not injured by soaking for one hour in either solution, fifteen minutes is undoubtedly

sufficient to accomplish the disinfection and there is no necessity for taking any risk on a longer treatment.

In using the corrosive sublimate solution it should be borne in mind that it is a strong poison ; also, that it readily corrodes metal, hence should not be used in metal vessels. The formalin solution is much less poisonous and does not corrode metal.

A convenient method of treating the seed is to place it in a small bag made of any loose cloth readily penetrated by water and suspend the bag in the disinfecting solution for the required length of time. The seed should be dried without delay. If artificial heat is used in drying great care should be taken to avoid over heating as the germination may be thereby injured. Probably drying the seed in the sun should be avoided as it is believed by some seedsmen that exposure to sunlight injures the germination of cabbage seed.

THE RELATION OF INFECTION ON THE SEED TO OUTBREAKS OF BLACK ROT.

On accepting the truth of the facts here presented regarding the danger of spreading *P. campestris* by means of the seed the reader should not conclude that a treatment of the seed before planting will insure total immunity from an outbreak of black rot. Neither should the absence of an outbreak in untreated seed be taken as a proof of the absence of disease germs. The presence of *P. campestris* is absolutely necessary, but it is only one of several factors concerned in such an outbreak. In addition to the presence of the disease germ there must be an available avenue of entrance to the host plant and the host plant itself must be in a favorable condition to succumb before the outbreak will occur.

Outbreaks of black rot in the seed beds are not frequent. In our own observations we have found diseased plants there only occasionally. However, by the time the disease appears in the field the seed beds are usually destroyed and our observations on this point have been correspondingly limited. From the early history of the fields which we have seen and from the observations of large growers of cabbage it seems probable that in exceptional cases many of the plants in a seed bed are infected

with *P. campestris*. In the absence of more direct observation, a discussion of the method of entrance of the germs to the plants in the seed bed has little value.

A second danger which probably is more important lies in the infection of the soil. By the act of transplanting, many of the plant roots are broken, exposing the cut ends of the fibro-vascular bundles. Some growers regularly break the ends from the tap roots when they are not accidentally removed. At this time if *P. campestris* is in the soil either of the seed bed or the field it has an opportunity to enter these broken bundles. Field observations have shown that infection through the roots is not infrequent. If the conditions are such as to favor an outbreak these root-infected plants quickly become a total loss and act as centers for spreading the disease in the field.

The disinfection of cabbage seed, like the treatment of seed potatoes is intended to prevent the spread of the disease organisms. When such treated seed is planted or the plants set in infected soil the treatment will have little value.

It is not expected that seed disinfection will give complete protection against black rot. In many cases the benefit may be very small. But as the treatment costs nothing except a little bother, and no other remedy is known, it is certainly advisable to disinfect the seed and thereby avoid unnecessary risk.